Carotenoid Pigments and β-Carotene in Paprika Fruits (*Capsicum* Spp.) with Different Genotypes[†]

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Recent evidence for antitumor activity of carotenoids in humans has revived interest in these compounds in foods, nutrition, and medicine. A wide variation in carotenoid content was found among various *Capsicum* genotypes. The total carotenoid content was generally higher in *C. annuum* lines than in the accessions from other species. In contrast with the wide variation found in the content of the major carotenoids, the proportions of individual carotenoids within the total content and the ratios among them showed much less variation. The coefficients of variation for the ratios ranged from 8 to 35% in comparison with 70–90% for the carotenoid content. Significant correlations were found among the concentrations of the major carotenoids. Increasing the carotenoid concentration in high-pigment fruits of paprika by genetic manipulation improved not only the quality of the fruit as a source of food colorant but also its nutritive value. The breeding line 4126 contains about 240 mg of carotenoids/100 g of fresh weight, of which 20 mg is β-carotene.

Keywords: *Carotenoids; β-carotene; high pigments; Capsicum spp.; genotypes*

INTRODUCTION

Red pepper fruits, especially from paprika cultivars, are used in the form of powders and oleoresins as spices and food colorants. These products are very rich in carotenoids, some of them specific to pepper fruits. The ketocarotenoids, capsanthin and capsorubin, occur only in red peppers and contribute to the red color (Philip *et al.*, 1971), whereas β-carotene, zeaxanthin, lutein, and β-cryptoxanthin are responsible for the yellow-orange color. Variation in the concentration of the carotenoids has been reported for various cultivars of paprika (Almela *et al.*, 1991); red-maturing varieties have been found to have higher carotenoid contents than yellow- or orange-maturing ones (Davies *et al.*, 1970), and a very high concentration has been found in black paprika (Deli *et al.*, 1992).

Accumulating evidence for the antitumor effect of carotenoids has focused a renewed interest onto these compounds (Poppel, 1993): some researchers have believed that these compounds may express their biological activity by contributing to the antioxidative defensive system of the organism (Krinsky, 1988), but, more recently, it has been found that the anticarcinogenic activity of retinoids and carotenoids is closely related to the enhancement of cell-to-cell communication (Zhang *et al.*, 1991).

The purpose of the present study was to estimate the variation in the content of major carotenoids and β-carotene in *Capsicum annuum* and other *Capsicum* species and to evaluate the biosynthetic linkage among the carotenoids of paprika fruits. The implications of the findings for the breeding of cultivars rich in carotenoids and β-carotene are discussed.

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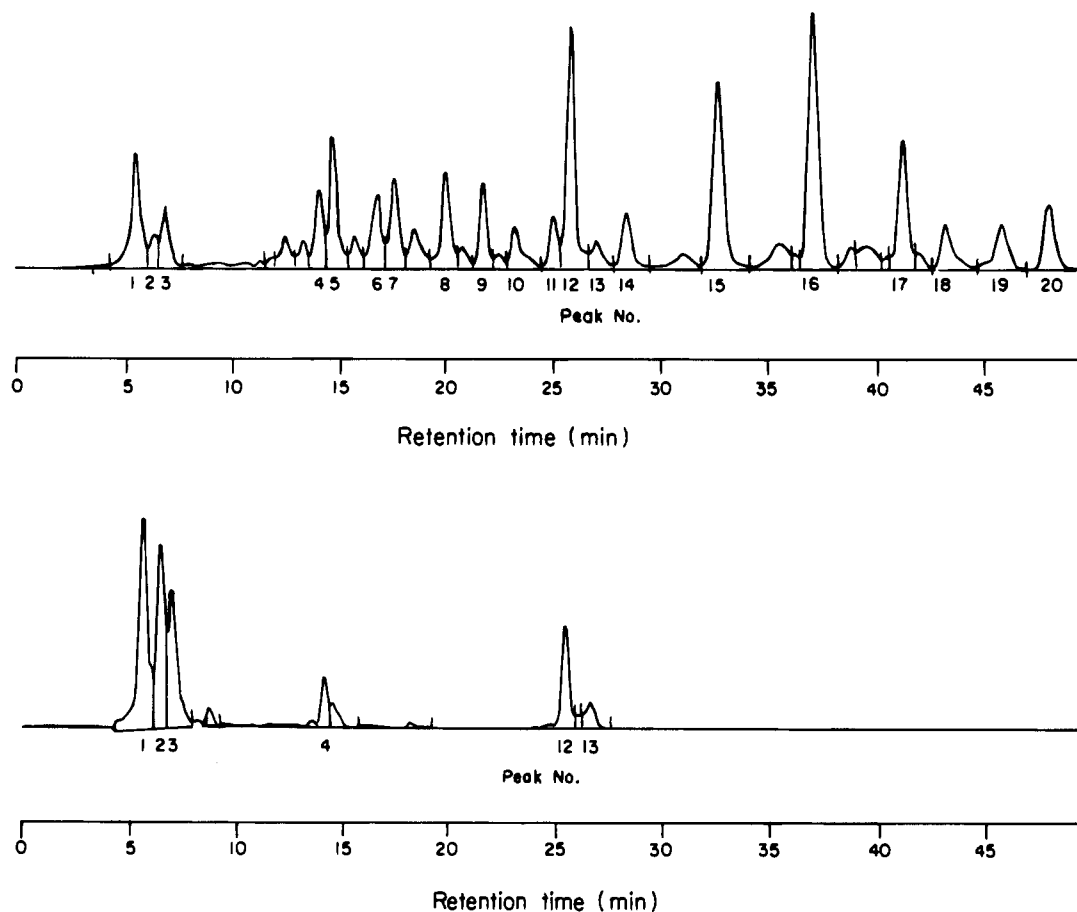‡ Department of Plant Genetics.
§ Department of Food Science.

MATERIALS AND METHODS

Materials. Seeds of *Capsicum baccatum, Capsicum chacoense, Capsicum chinense,* and *Capsicum frutescens* were obtained from Prof. Bosland (New Mexico State University, Las Cruces, NM) and planted in a glasshouse; controlled selfing of individual plants from each species was practiced. The progenies were sown in the field simultaneously with 15 different breeding lines of paprika, which were selected from segregating populations of several hundreds of plants analyzed for carotenoid content to provide a wide variation in the carotenoid content of the fruits. From each genotype, a bulk of fully mature fruits (at the start of dehydration), comprising 20–30 plants, was harvested. The fruits were dried at 45 °C for 72 h in darkness and then ground and sieved (1 mm mesh size) to give a fine powder. The samples were stored at −20 °C pending chemical analysis. All chemicals used were of ACS grade, purchased from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO), and all solvents used in chromatography were of HPLC grade.

Methods. *Pigment Extraction.* In the complete absence of light, pigments of paprika were extracted by shaking 50 mg of powder with 50 mL of acetone for 10 min. The mixture was left to stand at room temperature for 18 h in the presence of 0.5% BHT. An aliquot (5 mL) was filtered, evaporated to dryness, redissolved in 200 μL of eluent, passed through a 0.45-μm filter, and injected (25 μL) into the HPLC column.

Hydrolysis Procedures. Paprika powder (500 mg) was suspended in a 2% BHT solution in 7.5 mL of absolute ethanol, and a 60% aqueous KOH solution (1.25 mL) was added. This suspension was bubbled with nitrogen at 60 °C for 25 min and chilled immediately thereafter in ice for 10 min. Water (5 mL) was then added, and the pigments were extracted repeatedly with 5-mL portions of hexane until no color could be observed in the extract. The hexane extracts were combined, washed with water (25 mL), and dried over anhydrous sodium sulfate. An aliquot for HPLC injection was prepared as described above.

Chromatographic Methods. High-performance liquid chromatography (HPLC) was conducted on a Hewlett-Packard HP 1090 liquid chromatograph equipped with a 1040 HPC diode array detector. Photodiode array measurements of spectral properties for the individual peaks (from 260 to 540 nm) were determined at the upslope, apex, and downslope. The fitting of the three spectra indicated the degree of peak purity. A

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Figure 1. Reversed-phase HPLC chromatogram of unsaponified (a, top) and saponified (b, bottom) extracts of carotenoid pigments from mature fruits of the paprika cultivar Lehava.

serially connected, end-capped octadecylsilane column (Merck RP-18e, 3.4 × 250 mm, 5-μm particles) and an octadecylsilane column (Merck RP-8, 3.4 × 12.5 mm, 5-μm particles) were used for HPLC separations. A guard column (RP-18, 1 cm) was also used. The pigments were eluted with a mixture of solution A (40:60 v/v, acetonitrile/2-propanol) and solution B (water) at a flow rate of 0.80 mL/min, using a solution B gradient of 14 to 0% in 40 min, as described previously (Ittah et al., 1993). Total carotenoids were estimated, as described by Benedek (1958); for calculating, an extinction coefficient of capsanthin 1% = 1905 was used (Dawson et al., 1969). For peak identification, the R_f values and absorption spectra were compared with those of standard material obtained from Sigma for β-carotene, zeaxanthin, and lutein and, after TLC separation, for capsanthin, capsorubin, and β-cryptoxanthin (Vinkler and Richter, 1972).

RESULTS AND DISCUSSION

The chromatogram of the carotenoids contained in an unsaponified extract of paprika (var. Lehava) is shown in Figure 1a. Over 30 peaks were detected at 460 nm, all having the characteristic UV–visible pattern of carotenoids. A fairly good fit of the spectra taken by the photodiode array detector for each peak indicates its purity and, therefore, the efficiency of the chromatography method we used. The more polar carotenoids, the nonesterified pigments, eluted first in a well-separated group, with retention time of 4–8 min followed sequentially by the less polar monoesters and by β-cryptoxanthin and β-carotene, with retention time of 10–28 min and, finally, the nonpolar diesters.

The chromatogram was divided into 20 major peaks identified as follows: 1 = capsanthin + capsorubin; 2 = unidentified ketocarotenoids; 3 = zeaxanthin + lutein;

4 = β-cryptoxanthin; 5–7 = monoesters of capsanthin and capsorubin; 8–10 = monoesters of zeaxanthin and lutein; 11 = unidentified; 12 = all-trans-β-carotene; 13 = 13-cis-β-carotene; 14–17, 19, 20 = diesters of capsanthin and capsorubin; 18 = diester of lutein.

The chromatogram of saponified carotenoids is shown in Figure 1b. It includes only seven major peaks: peaks 1–3 are identical to the peaks in chromatogram 1a. Peak 4 is β-cryptoxanthin, and the small peak at RT 14.4 is the 13-cis isomer of β-cryptoxanthin. Peaks 12 and 13 are all-trans-β-carotene and its 13-cis isomer.

The major carotenoid contents of the fruits are presented in Table 1. A large variation in the carotenoid content was found among the several genotypes. The total carotenoid content was generally higher in *C. annuum* lines than in the accessions from other species, ranging from 390 to 16 600 μg/g. In all genotypes, most of the red pigments were esterified and only a small proportion of them was free; furthermore, diesters were generally predominant over monoesters. These results extend the findings reported for the paprika variety Sz-20 (Biacs et al., 1989) and for red pepper cultivars (Almela et al., 1991) to other *Capsicum* species and populations. The variation among the *C. annuum* lines is more important for breeding cultivars rich in carotenoids than among the other species. However, additional accessions of the related species should be analyzed for a more appropriate comparison of the populations.

In contrast with the large variation found in the content of the major carotenoids, the proportion of individual carotenoids within the total content and the ratios between them showed only a reduced variation

Table 1. Carotenoid Content (Micrograms per Gram of Dry Weight) in the Fruits of Various Species and Lines of *Capsicum*

line no.	species	total carotenoids	free capsanthin + capsorubin	free zeaxanthin + lutein	*trans*-β-carotene	*cis*-β-carotene	monoesters	diesters
4002	*C. baccatum*	1420	60	40	126	35	296	275
4005	*C. chinense*	4000	144	68	288	108	960	1172
4015	*C. frutescens*	1310	37	14	117	45	225	327
4017	*C. chacoense*	430	21	8	26	12	83	123
4018	*C. chacocense*	1000	52	17	81	31	195	227
4019	*C. chacoense*	390	14	5	28	13	70	100
4101	*C. annuum*	3250	117	55	254	88	672	862
4105	*C. annuum*	8790	659	281	580	211	1881	1881
4107	*C. annuum*	8070	331	153	581	234	1614	2212
4108	*C. annuum*	2950	103	50	145	62	589	856
4113	*C. annuum*	3570	182	75	136	68	700	996
4115	*C. annuum*	7900	561	213	419	166	1596	1968
4116	*C. annuum*	7460	216	104	455	209	1492	2373
4117	*C. annuum*	5480	137	77	345	170	1008	1557
4119	*C. annuum*	8850	566	212	681	257	2106	2125
4123	*C. annuum*	10710	396	193	728	311	2334	2828
4126	*C. annuum*	16600	714	299	1643	614	3518	3403
4128	*C. annuum*	10370	581	218	840	332	2178	2374
4135	*C. annuum*	8040	249	113	410	193	1719	2420
4136	*C. annuum*	7360	132	66	493	177	1486	2421
4140	*C. annuum*	5670	187	108	323	85	1203	1916
	mean	5886.7	260.0	112.8	414.2	162.9	1234.5	1543.6
	SD	4140.8	227.0	90.3	368.2	141.5	892.7	988.4
	range	390–16600	14–714	5–299	26–1643	12–614	70–3518	100–3403
	CV	70.3	87.3	80.0	88.9	86.8	72.3	64.0

Table 2. Mean Ratios between the Carotenoids in Fruits of Different Species of *Capsicum*

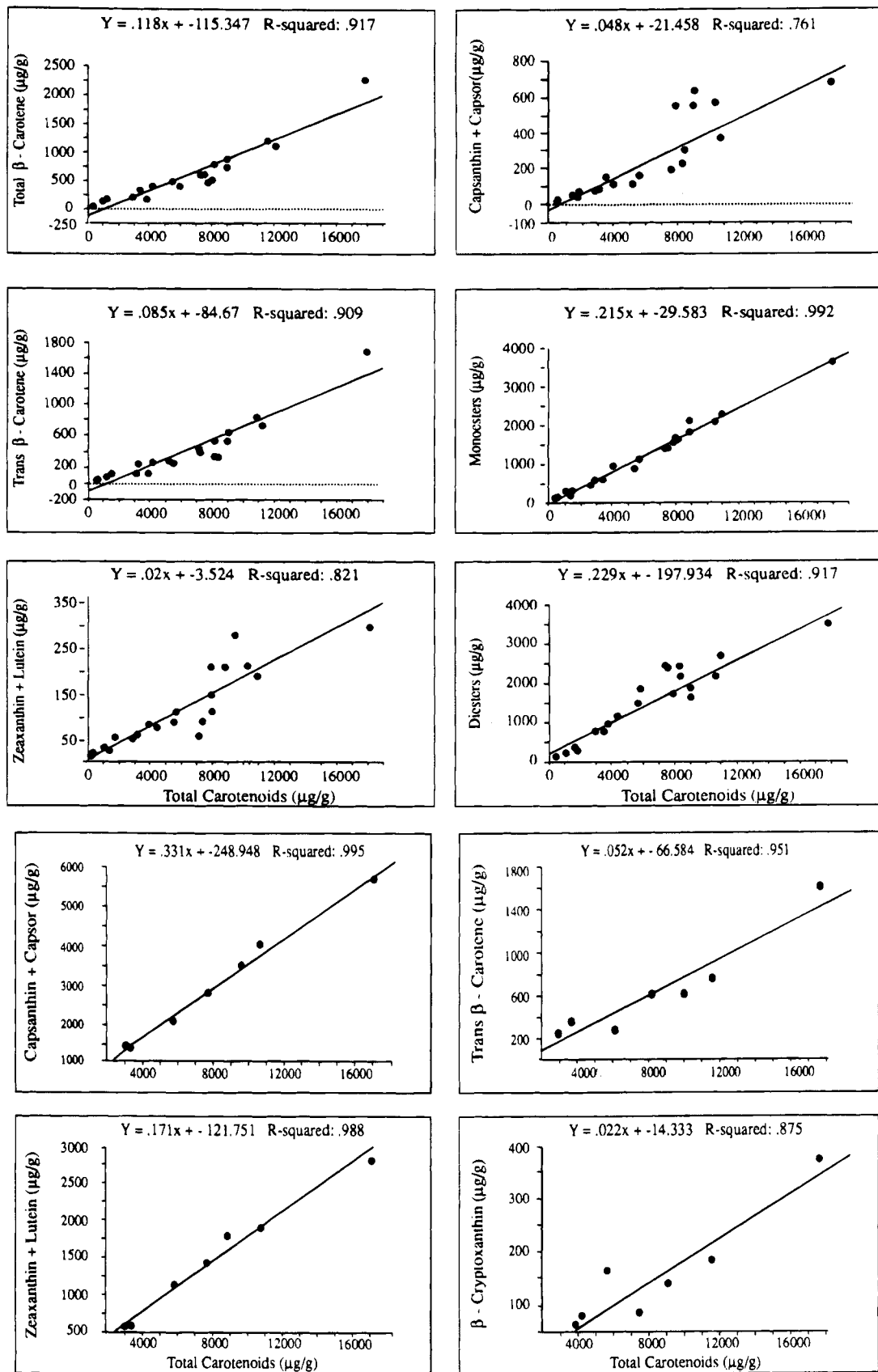|	capsanthin + capsor/total	zeaxanthin + lutein/total	β-carotene/total	*cis*-β-carotene/total	monoester/total	diester/total	monoester/diester	*cis/trans* β-carotene
mean	0.042	0.018	0.069	0.027	0.205	0.266	0.787	0.400
SD	0.015	0.006	0.015	0.005	0.016	0.04	0.144	0.063
range	0.018–0.075	0.009–0.032	0.038–0.099	0.015–0.037	0.172–0.24	0.194–0.338	0.614–1.076	0.263–0.5
CV	35.3	30.7	21.6	19.8	8.0	14.9	18.2	15.6

(Table 2). The coefficients of variation (CV) of the ratios ranged from 8.0 to 35% in comparison with 70–90% for the carotenoid content. Similar results have been reported for a few cultivars of *C. annuum* (Minguez-Mosquera *et al.*, 1992). The narrow range of variation among the ratios of the carotenoids is the first piece of evidence for the linkage between the levels of synthesis and accumulation of the various major carotenoids in the fruit. Further evidence was obtained from the regression analysis of the various carotenoid contents. High, significant regression and correlation coefficients were found between the total carotenoid content and the concentration of each major carotenoid or its esters (Figure 2a). Significant correlations were also found among the concentrations of individual carotenoids or their esters. Furthermore, the carotenoid concentrations remained significantly correlated after hydrolysis (Figure 2b). The genetic component of these correlation coefficients is high since the lines and species were from distant genetic backgrounds.

The established pathway of the biosynthesis of keto-carotenoids in *C. annuum* fruits shows that β-carotene, β-cryptoxanthin, and most probably zeaxanthin are precursors of cryptocapsin, capsanthin, and capsorubin (Camara, 1980; Ruttiman, 1982). The significant positive correlation among the various carotenoid contents is indicative of genetic linkage or pleiotropy between genes governing the synthesis of different carotenoids in the pathway. The interpretation of these results, at the level of biosynthesis and accumulation of the major carotenoids in the fruit, is that the whole biosynthetic pathway is regulated at different levels in different genotypes; i.e., the concentrations of the precursors and

also of the carotenoids along the pathway are determined by common regulatory genes. Indeed, our results show that genotypes with high total carotenoid content display a high concentration of the major carotenoids in the fruit and *vice versa*. The very low carotenoid content in yellow- and orange-maturing fruits (Davies *et al.*, 1970) and the positive correlation reported between some precursors of the red pigments (Almela *et al.*, 1991) form additional supporting evidence for this interpretation.

These findings have important implications for the breeding and development of paprika cultivars with a high content of red pigments, which is the most important quality criterion of the oleoresin and powder (Kanner *et al.*, 1978; Bosland, 1993). The linkage among the concentrations of the major fruit carotenoids might limit the prospects of breeding paprika cultivars with substantial qualitative differences in the ratios of these major carotenoids. Likewise, the selection of plants with increased capsanthin or capsorubin content should result in increased contents of other carotenoids, such as β-carotene. This compound can substitute for the red pigments as a selection criterion for increasing the color intensity of the fruit. Another alternative would be to screen segregating populations for high total carotenoid content.

Increasing the carotenoid concentration in high-pigment fruits of red pepper by genetic manipulation seems to improve not only the quality of the fruit as a food colorant but also its importance as an excellent source of β-carotene and total carotenoids. The breeding line 4126 contains about 240 mg of carotenoids/100 g of fresh weight, of which 20 mg is β-carotene. The amount

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Figure 2. Regression analysis between total carotenoids and the content of the major carotenoids before (a, top six panels) and after saponification (b, bottom four panels) of 21 or 7 lines of paprika fruits from different *Capsicum* species.

of β-carotene in this line of paprika is comparable with that found in carrots, but the total carotenoid content is 6 times higher than that of carrots, so that this fruit seems to be one of the richest known plant sources of

carotenoids (Mangels *et al.*, 1993). Increasing the amount of the carotenoids in paprika fruits should increase their value not only as food colorants but also as an important nutritional source.

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